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Serum Vascular Endothelial Growth Factor Predicts Venous Invasion in Hepatocellular Carcinoma: A Prospective Study

Ronnie Tung-Ping Poon, MS, FRCS (Edin)* Irene Oi-Lin Ng, MD, FRCPath† Cecilia Lau, BMLS* Li-Xin Zhu, PhD* Wan-Ching Yu, MBBS* Chung-Mau Lo, MS, FRCS (Edin), FRACS* Sheung-Tat Fan, MS, MD, FRCS (Edin & Glasg), FACS* and John Wong, PhD, FRACS, FACS*

*From the Centre of Liver Diseases, Departments of *Surgery and †Pathology, The University of Hong Kong, Queen Mary Hospital, Hong Kong, China*

Objective

To evaluate the correlation between serum vascular endothelial growth factor (VEGF) level and the clinicopathologic features in patients with hepatocellular carcinoma (HCC).

Summary Background Data

VEGF is an important angiogenic factor regulating tumor angiogenesis. A high serum VEGF level has been shown to be associated with tumor progression and metastasis in several human cancers, but its significance in HCC is unclear. The correlation between serum VEGF level and tumor pathologic features in patients with HCC has not been studied before.

Methods

Preoperative serum samples and tumor specimens were prospectively collected in 100 patients undergoing resection of HCC. Serum VEGF level was measured by enzyme-linked immunosorbent assay, and tumor VEGF expression was assessed by immunohistochemical study. Histopathologic examination was performed by a pathologist without prior knowledge of the serum VEGF level or tumor VEGF expression.

Results

Preoperative serum VEGF levels ranged from 15 to 1,789 pg/mL (median 269). When serum VEGF levels were compared between groups categorized by different clinicopathologic variables, significant correlation was found between a high serum VEGF level and absence of tumor capsule, presence of intrahepatic metastasis, presence of microscopic venous invasion, and advanced stage. There was a positive correlation between the serum VEGF level and tumor expression of VEGF as well as platelet count. When the 75th percentile serum VEGF level (500 pg/mL) was used as a cutoff level, the frequency of venous invasion in patients with a high serum VEGF level was significantly greater compared with patients with a low serum VEGF level. By multivariate analysis, a serum VEGF level of more than 500 pg/mL and tumor size more than 5 cm were independent preoperative factors predictive of microscopic venous invasion. During a median follow-up of 11.6 months, 48% of patients with a serum VEGF level of more than 500 pg/mL and 27% of those with a serum VEGF level of 500 pg/mL or less developed postoperative recurrence.

Conclusions

These results show that a high preoperative serum VEGF level is a predictor of microscopic venous invasion in HCC, suggesting that the serum VEGF level may be useful as a biologic marker of tumor invasiveness and a prognostic factor in HCC.

Hepatocellular carcinoma (HCC) is a highly vascular tumor characterized by neovascularization and a high propensity for venous invasion. Neovascularization involves sprouting of new blood vessels from preexisting ones, a process also known as angiogenesis. Angiogenesis is essential for tumor growth, invasion, and metastasis.[1,2] The neovasculature facilitates shedding of tumor cells into surrounding blood vessels.[1] However, the exact mechanism that results in frequent vascular invasion in HCC remains unclear.

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Correspondence: Dr. Ronnie Tung-Ping Poon, Department of Surgery, Queen Mary Hospital, 102 Pokfulam Rd., Hong Kong, China.
E-mail: poontp@hkucc.hku.hk
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Vascular endothelial growth factor (VEGF) is the most potent directly acting angiogenic factor known so far.[3] It is a soluble, dimeric 46-kd glycoprotein that specifically stimulates endothelial cell proliferation and enhances vascular permeability.[3] It is secreted by various human cancers, including HCC.[4–10] VEGF expression by tumors is closely related to tumor progression and prognosis, and it may be an important factor in tumor metastasis.[6,7,11–13] A few studies have reported that increased expression of VEGF might be associated with venous invasion and metastasis in HCC.[14–16]

Recently, serum concentrations of VEGF have been examined in patients with various histologic types of cancer, and elevated serum VEGF levels have been observed in patients with disseminated cancer compared with those with localized disease.[17–20] Several studies have revealed a predictive value of the serum VEGF level for disease progression and prognosis in cancers of different origins, including the breast,[17] colon,[18] kidney,[21] urothelium,[22] ovary,[23] lung,[24] and lymphoma.[25] However, little is known of the clinical significance of the serum VEGF level in HCC. The results of a recent study showed that circulating VEGF levels were markedly elevated in HCC patients with remote metastasis compared with those without metastasis, suggesting that circulating VEGF may be a marker for metastasis in HCC.[26] It may be more useful clinically if the serum VEGF level could predict early microscopic vascular invasion in HCC. To our knowledge, no studies have evaluated the correlation between the serum VEGF level and histopathologic features of HCC. A prospective study was therefore conducted to evaluate the relation between preoperative serum VEGF levels and various clinicopathologic parameters in 100 patients undergoing resection for HCC, and in particular to investigate whether the serum VEGF level is a predictive factor of venous invasion in HCC.

METHODS

Patients and Samples

Between January 1998 and August 1999, 100 consecutive patients (76 men, 24 women, median age 57, range 23–79) underwent curative resection for HCC, defined as macroscopically complete removal of the tumor, in the Department of Surgery of the University of Hong Kong at Queen Mary Hospital. The criteria for resectability were absence of distant metastasis, absence of main portal vein thrombosis, anatomically resectable disease, and adequate liver function reserve. None of the patients received any preoperative treatment.

Preoperative serum samples were prospectively collected from these patients. Venous blood samples were drawn into a serum separator tube and centrifuged at 3,000 rpm for 10 minutes, then stored at −80°C until VEGF levels were determined. To determine any correlation between the serum VEGF level and tumor expression of VEGF, fresh tumor specimens were obtained in the operating room immediately after resection, fixed in formalin, and then embedded in paraffin for immunohistochemical staining of VEGF expression. The main surgical specimens were sent to the department of pathology for conventional histopathologic study.

Serum samples were obtained from 20 healthy adults (14 men, 6 women, median age 55, range 24–70 years) with no evidence of active disease as controls for assay of serum VEGF levels.

Measurement of Serum VEGF Level

Serum VEGF levels were quantitatively measured by an enzyme-linked immunosorbent assay kit designed to measure human VEGF concentration in serum (Quantikine Human VEGF Immunoassay; R & D Systems, Minneapolis, MN). This assay for the serum VEGF level has been shown to be reliable and reproducible in previous studies.[18–26] Briefly, 100 μL recombinant human VEGF standard and serum sample was serially diluted and pipetted into a microtiter plate coated with murine monoclonal antibody specific for human VEGF and incubated for 2 hours at room temperature. Any VEGF present was bound by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase-linked polyclonal antibody specific for VEGF was added to each well to sandwich the VEGF. After further washings to remove any unbound antibody–enzyme reagent, tetramethylbenzidine was added. The intensity of color developed, which was in proportion to the amount of VEGF bound, was measured by reading absorbance at 450 nm. Each measurement was made in duplicate, and the VEGF level was determined from a standard curve generated for each set of samples assayed. The sensitivity of the assay was 9 pg/mL, and the coefficients of variation of intraassay and interassay determinations were in the range given by the manufacturer (4.5–6.7% and 6.2–8.8%, respectively).

Immunohistochemical Study for Tumor VEGF Expression

Immunohistochemical staining for VEGF was performed on 4-μm-thick paraffin-embedded sections of tumorous tissues using the streptavidin-biotin-peroxidase complex technique. A mouse monoclonal antibody specific for human VEGF (VEGF [Ab3], Calbiochem, Oncogene Research Products, Cambridge, MA) was used at 1:800 dilution. The surrounding nontumorous liver served as an internal positive control. Negative control was obtained by replacing the primary antibody with nonimmunized rabbit serum.

A semiquantitative method was used to determine VEGF expression in HCC tissue. For each section, five random areas were selected in ×400 fields, and the number of tumor cells with positive or negative staining for VEGF was counted with the help of a computer image analysis system

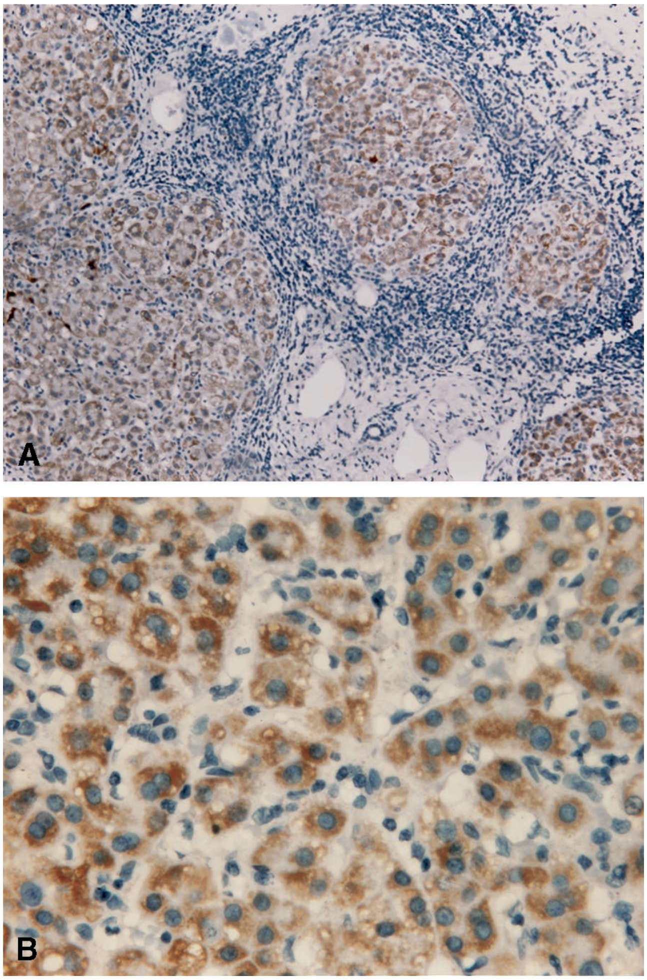

Figure 1. Immunostaining showing positive staining for vascular endothelial growth factor (VEGF; brownish staining) in the cytoplasm of the majority of tumor cells in a tumor specimen with high VEGF expression. (A) ×100. (B) ×400.

(MetaMorph Imaging System Version 3.0; Universal Imaging Corp., West Chester, PA). The percentage of cells with positive staining for each tumor was calculated by averaging the results of five counts. Tumors were classified into high (positive staining for VEGF in >50% of cells) and low (positive staining for VEGF in 50% or fewer cells) VEGF expression. Figure 1 shows an example of a tumor with high VEGF expression. The procedure of immunohistochemical study was performed by a single investigator without prior knowledge of the serum VEGF level or clinicopathologic data.

Clinicopathologic Data

Preoperative clinical and laboratory data including imaging findings, routine liver biochemistry, complete blood count, indocyanine green retention at 15 minutes (ICG_{15}), hepatitis B surface antigen status, and serum alpha fetoprotein (AFP) level were prospectively collected for each patient in a computerized database.

Histopathologic examination of all specimens was performed by a senior pathologist with experience in HCC pathology who was unaware of the preoperative serum VEGF level or tumor VEGF immunostaining results. Tumors were graded according to the criteria described by Edmonson and Steiner.[27] Staging was performed using the International Union Against Cancer (UICC) pathologic tumor-node-metastasis (pTNM) classification.[28] Serial sections of the tumor and the surrounding liver were carefully examined to identify any microscopic venous invasion or microsatellite nodules surrounding the main tumor, the latter being regarded as intrahepatic metastasis.[29]

Statistical Analysis

Serum VEGF levels were compared between groups categorized by various clinicopathologic features using the Mann-Whitney test. Correlation between continuous variables was evaluated by the Spearman correlation coefficient (r). The chi-square test with Yates correction (or the Fisher test where appropriate) was used to compare the incidence of microscopic venous invasion between groups categorized by different preoperative parameters, including the preoperative serum VEGF level. A logistic regression analysis was used to identify independent preoperative factors predictive of microscopic venous invasion. Cumulative disease-free survival curves were computed using the Kaplan-Meier method and compared between patients with high and low serum VEGF levels by the log-rank test. All statistical analyses were performed using computer software (SPSS/PC+; SPSS Inc., Chicago, IL). $P < .05$ was considered statistically significant.

RESULTS

Correlation Between Preoperative Serum VEGF Level and Clinicopathologic Features

The preoperative median serum VEGF level in the patients with HCC was 269 pg/mL (range 15–1,789), significantly higher than the median of 180 pg/mL in 20 healthy control subjects (range 41–671) ($P = .048$). The range of serum VEGF levels in the control subjects in this study was compatible to that described by R & D Systems in healthy human serum samples (62–707 pg/mL).

There was no significant association between preoperative serum VEGF levels and various clinical and laboratory variables (Table 1), except the platelet count. Patients with a platelet count of more than 150×10^9 /L had significantly higher serum VEGF levels compared with those with a platelet count of less than 150×10^9/L. There was a significant correlation between the serum VEGF level and platelet number ($r = 0.432$, $P = .001$) (Fig. 2).

Table 2 shows the preoperative serum VEGF levels of patients categorized by various pathologic features. There

Table 1. PREOPERATIVE SERUM VEGF LEVELS OF PATIENTS CATEGORIZED BY CLINICAL AND LABORATORY VARIABLES

	Median Serum VEGF Level (pg/mL)	*P* Value
Gender		
Male (n = 76)	239	.110
Female (n = 24)	348	
Age		
≤50 years (n = 33)	267	.971
>50 years (n = 67)	247	
HBsAg		
Positive (n = 83)	267	.927
Negative (n = 17)	247	
Serum AFP		
≤20 ng/mL (n = 36)	225	.166
>20 ng/mL (n = 64)	288	
ICG_{15}		
≤10% (n = 44)	285	.582
>10% (n = 56)	235	
Serum albumin		
≤40 g/L (n = 59)	257	.970
>40 g/L (n = 41)	270	
Serum AST		
≤50 u/L (n = 68)	247	.376
>50 u/L (n = 32)	346	
Serum ALT		
≤50 u/L (n = 62)	246	.657
>50 u/L (n = 38)	311	
Platelet count		
$\leq 150 \times 10^9$/L (n = 44)	172	.001
$>150 \times 10^9$/L (n = 56)	351	

VEGF, vascular endothelial growth factor; HBsAg, hepatitis B surface antigen; AFP, alpha-fetoprotein; ICG_{15}, indocyanine green retention at 15 minutes; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 2. PREOPERATIVE SERUM VEGF LEVELS OF PATIENTS CATEGORIZED BY PATHOLOGIC VARIABLES

	Median Serum VEGF Level (pg/mL)	*P* Value
Cirrhotic liver		
No (n = 52)	287	.694
Yes (n = 48)	217	
Tumor size		
≤5 cm (n = 42)	220	.096
>5 cm (n = 58)	301	
Edmonson grade		
1/2 (n = 51)	235	.272
3/4 (n = 49)	316	
Tumor capsule		
Present (n = 39)	214	.023
Absent (n = 61)	346	
Venous invasion		
Absent (n = 58)	215	<.001
Present (n = 42)	414	
Microsatellite nodules		
Absent (n = 59)	239	.019
Present (n = 41)	355	
pTNM stage		
I/II (n = 49)	214	.001
IIIA/IVA (n = 51)	368	

VEGF, vascular endothelial growth factor; pTNM, pathologic tumor-node-metastasis.

was no significant association between the serum VEGF level and the presence of cirrhosis, the size of the tumor, or the histologic grade. Significantly greater serum VEGF levels were associated with the absence of tumor capsule, the presence of microscopic venous invasion, the presence of microsatellite nodules, and an advanced pTNM stage. Serum VEGF levels increased significantly with advancing tumor pTNM stage (Fig. 3). The serum VEGF levels in patients with stage IIIA or IVA disease were significantly greater than those of normal control subjects, whereas the serum VEGF levels in patients with stage I or II disease were not. Figure 3 shows that there was also an increasing trend of platelet count with advancing tumor pTNM stage. Figure 4 shows that there was a positive correlation between serum VEGF levels and tumor VEGF expression as assessed by immunohistochemical study (P = .043).

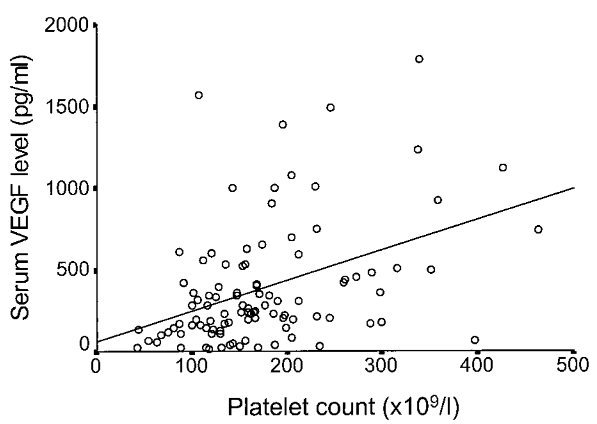

Figure 2. Scatter plot showing the correlation between the serum level of vascular endothelial growth factor (VEGF) and platelet count (r = 0.432, P = .001).

Preoperative Serum VEGF Level as a Predictor of Venous Invasion

A further analysis was performed to investigate whether the preoperative serum VEGF level was an independent factor predictive of venous invasion in HCC among various other preoperative clinical, radiologic, and laboratory parameters. Because of a skewed distribution of serum VEGF levels, the 75th percentile level (500 pg/mL) in the 100 HCC patients was defined as the cutoff value for high and low serum VEGF levels in this analysis.[30] This serum VEGF level (500 pg/mL) corresponded to the 95th percentile value in the normal control subjects described by R & D

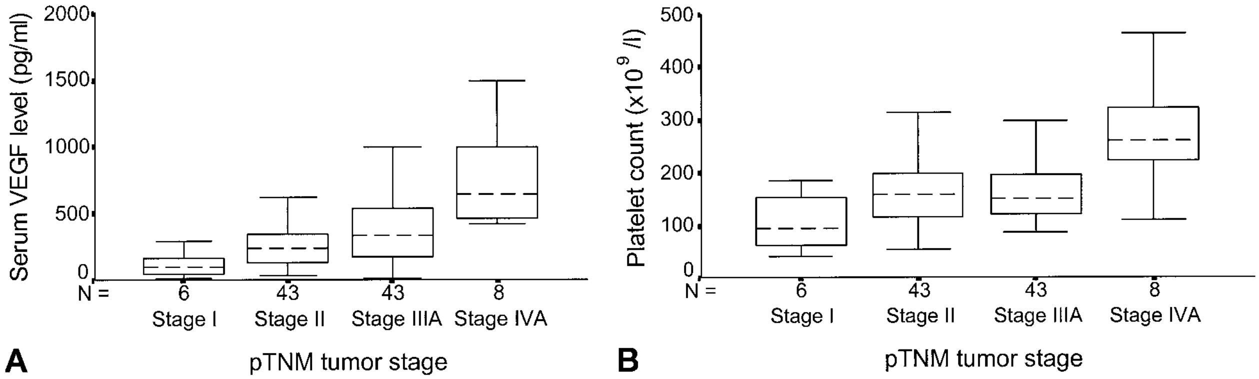

Figure 3. (A) Serum levels of vascular endothelial growth factor (VEGF) in patients with tumors of different stages. Values in each group were significantly different from the other groups ($P < .05$). The upper and lower quartiles and the median values (dotted line) are depicted as box plots. Error bars indicate data within 1.5 times the interquartile range. (B) Platelet count in patients with tumors of different stages. Values in the stage IVA group were significantly greater than values in the stage I, II, or IIIA groups ($P < .05$), but there were no significant differences between other tumor stages.

Systems, which was used as the cutoff value of elevated serum VEGF in cancer patients in another study.[18]

Table 3 shows the results of univariate analysis. A tumor size of more than 5 cm was associated with a higher incidence of microscopic venous invasion. Radiologic evidence of portal vein thrombosis on computed tomography or arteriography was also correlated with microscopic venous invasion, but this feature was present in only in a few patients with microscopic venous invasion. A higher incidence of venous invasion was also noted in patients with an ICG_{15} of 10% or less. However, there was a higher proportion of large tumors (>5 cm) in patients with an ICG_{15} of 10% or less compared with those with an ICG_{15} of more than 10% (68% vs. 50%). Large tumors were more likely to be selected for resection if the liver function reserve was good. A serum VEGF level of more than 500 pg/mL was associated with a significantly higher incidence of venous invasion compared with a serum VEGF level of 500 pg/mL or less ($P < .001$). The frequency of venous invasion in patients with a platelet count of more than 150×10^9/L was greater than that of patients with a low platelet count (46% vs. 36%), but the difference was not statistically significant ($P = .113$).

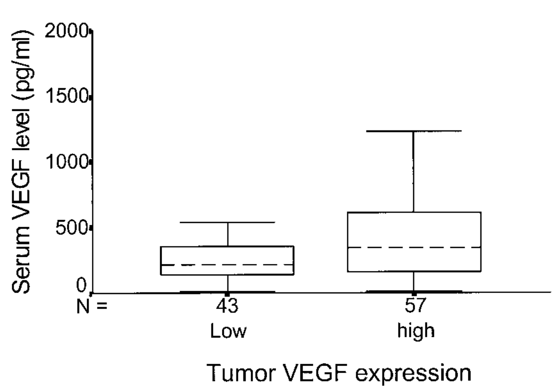

Figure 4. Correlation between serum levels of vascular endothelial growth factor (VEGF) and tumor expression of VEGF evaluated by immunohistochemical staining ($P = .043$). The upper and lower quartiles and the median values (dotted line) are depicted as box plots. Error bars indicate data within 1.5 times the interquartile range.

After correcting for confounding variables by multivariate analysis, only tumor size of more than 5 cm (risk ratio 5.770, 95% confidence interval 2.163–15.389, $P = .001$) and serum VEGF level of more than 500 pg/mL (risk ratio 3.068, 95% confidence interval 1.223–7.693, $P = .034$) were independent preoperative factors predictive of microscopic venous invasion. The positive predictive value for tumor size more than 5 cm was 59% (34/58), and the negative predictive value was 81% (34/42). The preoperative serum VEGF level of more than 500 pg/mL was associated with a positive predictive value of 72% (18/25) and a negative predictive value of 68% (51/75). Combining these two preoperative factors, patients with tumors more than 5 cm and serum VEGF levels of more than 500 pg/mL had an 88% (14/16) incidence of venous invasion, patients with tumors more than 5 cm but serum VEGF levels of 500 pg/mL or less had a 48% (20/42) incidence of venous invasion, patients with tumors 5 cm or less but serum VEGF levels more than 500 pg/mL had a 44% incidence of venous invasion (4/9), and patients with tumors 5 cm or less and serum VEGF levels of 500 pg/mL or less had a 12% (4/33) incidence of venous invasion.

Preoperative Serum VEGF Level and Postoperative Recurrence

All patients had regular follow-up and surveillance for recurrence by monthly serum alpha-fetoprotein level, chest x-ray, and ultrasound or computed tomography scan every 2 to 3 months. During a median follow-up of 11.6 months (range 2.5–21.4), 27% (20/75) of patients with serum VEGF

Table 3. UNIVARIATE ANALYSIS OF PARAMETERS PREDICTIVE OF MICROSCOPIC VENOUS INVASION

	Venous Invasion Negative (n = 58)	Venous Invasion Positive (n = 42)	*P* Value
Clinical Parameters			
Gender			
Male	43	33	.608
Female	15	9	
Age			
≤50 years	17	16	.356
>50 years	41	26	
HBsAg			
Positive	46	37	.248
Negative	12	5	
Radiologic Parameters			
Tumor size			
≤5 cm	34	8	<.001
>5 cm	24	34	
Portal vein thrombosis			
No	58	36	.004
Yes	0	6	
Tumor number			
Solitary	54	38	.198
Multiple	4	4	
Laboratory Parameters			
Serum AFP			
≤20 ng/mL	23	13	.371
>20 ng/mL	35	29	
ICG_{15}			
≤10%	20	24	.033
>10%	38	18	
Serum albumin			
≤40 g/L	35	24	.748
>40 g/L	23	18	
Serum AST			
≤50 u/L	43	25	.122
>50 u/L	15	17	
Serum ALT			
≤50 u/L	36	26	.797
>50 u/L	22	16	
Platelet count			
$\leq 150 \times 10^9$/L	28	16	.113
$>150 \times 10^9$/L	30	26	
Serum VEGF			
≤500 pg/mL	51	24	<.001
>500 pg/mL	7	18	

HBsAg, hepatitis B surface antigen; AFP, alpha-fetoprotein; ICG_{15}, indocyanine green retention at 15 minutes; AST, aspartate aminotransferase; ALT, alanine aminotransferase; VEGF, vascular endothelial growth factor.

levels of 500 pg/mL or less and 48% (12/25) of patients with serum VEGF levels of more than 500 pg/mL developed postoperative recurrence ($P = .048$). Figure 5 shows the cumulative disease-free survival curves of patients with preoperative serum VEGF less than or greater than 500 pg/mL. The 1-year disease-free survival rate was 61.5% in patients with serum VEGF levels of 500 pg/mL or less,

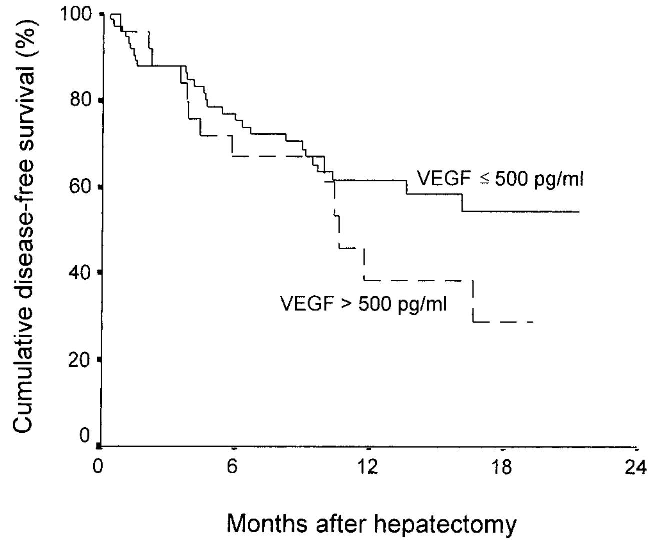

Figure 5. Cumulative disease-free survival curves of patients with serum levels of vascular endothelial growth factor (VEGF) less than and greater than 500 pg/mL ($P = .085$).

compared with 38.2% in those with serum VEGF levels of more than 500 pg/mL ($P = .085$).

DISCUSSION

Surgical resection is the mainstay of treatment with a hope of cure for patients with HCC. However, the prognosis after resection of HCC has remained unsatisfactory because of a high incidence of postoperative recurrence.[31–33] Most recurrences occur in the liver remnant rather than extrahepatic sites. Intrahepatic metastasis resulting from portal venous invasion is considered an important mechanism of intrahepatic recurrence.[32,33] Several studies have shown that microscopic venous invasion is the single most important prognostic factor for postoperative recurrence and survival after resection of HCC.[31–34] In recent years, liver transplantation has been accepted as a curative treatment for patients with small HCC (<5 cm), but the presence of microscopic venous invasion was also associated with an unfavorable outcome.[35–38] In a recent series of 71 liver transplants for HCC, microvascular invasion was associated with a 0% rate of 3-year tumor-free survival, whereas 94% of patients without microvascular invasion were tumor-free after 3 years.[36]

The exact mechanism of venous invasion in HCC remains unclear, but active neovascularization of the tumor is likely to play an important role. Our results show, for the first time, a strong correlation between a high preoperative serum VEGF level and the presence of microscopic venous invasion in HCC. A high serum VEGF level was also associated with the absence of tumor capsule and the presence of intrahepatic metastasis, both indicative of an invasive phenotype of the tumor.[29] Because VEGF is an important mediator of angiogenesis, these findings support a role of angiogenesis in vascular invasion in HCC.

The expression of VEGF has been found to be upregulated in many tumor types, including HCC.[10] An important source of circulating VEGF is presumably the tumor tissue.[39] Previous studies on serum VEGF in cancer patients have not investigated its relation with tumor expression of VEGF. In this study, there was a positive correlation between the serum VEGF level and the tumor VEGF expression evaluated by immunohistochemical staining, suggesting that the serum VEGF level at least in part reflects the tumor VEGF expression.

A positive correlation was also found between the serum VEGF level and the platelet count. This result is in accordance with findings reported by other authors.[26,39] Recent studies have shown that platelets have the function of storing circulating VEGF.[39–42] These studies have also shown that the storage and release of VEGF by circulating platelets may have an important role in processes involving platelet and endothelial cell interactions, such as wound healing and tumor invasion. It has been postulated that platelets adhering to circulating tumor cells may be activated to release VEGF at points of adhesion to endothelium, leading to hyperpermeability and extravasation of tumor cells.[42] There is also preliminary evidence suggesting that fast-growing tumors may release thrombopoietic cytokines in addition to angiogenic factors such as VEGF.[39] An increase in the number of circulating platelets, by uptake of free VEGF, may focus the effect of this angiogenic factor to sites where activation of platelet takes place—for example, a wound or a tumor.[39] Our data in fact show that the platelet count in patients with stage IVA disease was significantly greater than that of patients with less advanced tumor stages, and a previous study has reported a higher platelet count among patients with distant metastatic disease compared with those with HCC localized to the liver.[26] HCC has been shown to express thrombopoietin, which could be a mediator in inducing thrombocytosis.[43] Although there was a significant correlation between the serum VEGF level and the platelet count, serum VEGF level does not seem to be a simple reflection of the number of circulating platelets. Considerable variation in the individual VEGF load of platelets among cancer patients has been observed.[39] In our analysis, there was no significant association between platelet count and venous invasion, whereas the serum VEGF level turned out to be an independent predictive factor for venous invasion.

In line with the findings in studies of other cancers, the lower-range serum VEGF levels in HCC patients overlapped considerably with those of normal subjects. Hence, the serum VEGF level is not useful for diagnosis of HCC. In fact, the serum VEGF levels of patients with stage I and II tumors were not significantly different from those of normal subjects. However, the elevated serum VEGF level appears to be a marker of tumor invasiveness in HCC. Although the cutoff level for the best predictive value needs to be clarified with a study of a larger sample of patients, our data show that a serum VEGF level of more than 500 pg/mL is an independent predictor of vascular invasion of HCC. The results of a previous study suggested that circulating VEGF level might be useful as a marker of hematogenous spread in HCC patients with remote metastasis.[26] Our results show that even in patients with resectable HCC apparently localized to the liver, a high serum VEGF level correlated with a significantly increased incidence of microscopic vascular invasion. HCC is characterized by its propensity for vascular invasion, and tumor size of more than 5 cm has been known to be a predictor of vascular invasion in HCC.[44] However, as demonstrated by our data, vascular invasion is also present in a substantial proportion of patients with small tumors. Serum VEGF level was another predictive factor for venous invasion independent of tumor size. This novel finding not only provides insight into the role of angiogenesis in the mechanism of vascular invasion in HCC, but also suggests potentially important clinical applications of serum VEGF measurement in the management of patients with HCC.

Preoperative serum VEGF level may help in selecting patients with HCC of an invasive phenotype who may benefit from neoadjuvant therapy before surgical resection or transplantation to reduce the risk of recurrence. Preoperative transarterial chemoembolization is the main neoadjuvant therapy currently used in some centers, although its efficacy remains controversial.[45–47] Preoperative chemoembolization before hepatic resection may be more beneficial in certain subgroups of patients,[47] and the results of one study showed that it was effective in inducing necrosis of intrahepatic metastases and improving the survival in this subgroup of patients.[45] Preoperative chemoembolization, if used, may be more effective in selected patients with a high risk of vascular invasion and intrahepatic metastasis. Temporary portal venous embolization is another method that has been used to prevent intrahepatic venous dissemination of tumor cells during resection of HCC,[48] and it may also be more appropriate in patients with a predicted high risk of vascular invasion. In this context, serum VEGF level may be a useful preoperative parameter to predict the invasiveness of a tumor. In the future, effective antiangiogenic agents may be available for neoadjuvant therapy in patients with invasive tumors. Experimental evidence suggests that antiangiogenic therapy may suppress the growth of metastasis in HCC.[49] The serum VEGF level may be useful for monitoring the tumor response to such therapies. A close correlation between changes in the serum VEGF level and tumor response to treatment in renal cell carcinoma has been recently reported.[50]

Serum VEGF level may also assist in selecting the definitive treatment for apparently localized HCC. Apart from a tumor size of more than 5 cm, microscopic vascular invasion is another unfavorable factor in liver transplantation for HCC.[36–38] Microvascular invasion cannot be used as a selection criterion because it cannot be defined before surgery.[36] It has been suggested that the use of molecular markers for the prediction of vascular invasion and hematogenous spread may assist in the selection of patients with HCC for transplantation.[38] Serum VEGF level may be an appropriate candidate of such a biologic marker.

Serum VEGF level may be useful as a new prognostic factor in patients with HCC. With a limited follow-up, our data show that patients with a high serum VEGF level had a greater risk of early recurrence. There was a trend toward a worse disease-free survival after hepatectomy in patients with a serum VEGF level of more than 500 pg/mL, although the difference did not reach statistical significance. A longer follow-up is awaited to clarify the prognostic significance of the serum VEGF level. The serum VEGF level may be particularly useful in providing prognostic information in the subset of patients with small tumors ($<$5 cm). We are also measuring longitudinally the serum VEGF level at intervals after resection of HCC to investigate any correlation with recurrence of the disease. The results of a previous study in breast cancer showed an increase in the circulating VEGF level associated with relapse of disease.[17]

In conclusion, this prospective study shows that the serum VEGF level is an independent factor predictive of microscopic venous invasion in HCC, suggesting that it may be a useful serum marker of tumor invasiveness in HCC. Further studies are required to evaluate its potential clinical applications in the management of patients with HCC.

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